REMARKS

Claims 1-19 are pending.

Claim Rejections Under 35 USC §112, second paragraph

Claims 1-19 stand rejected as allegedly failing to set forth as the subject matter which Applicants regard as their invention. Particularly, the action at paragraphs 1-6 rejects various claims under 35 USC §112, second paragraph. With respect to paragraphs 2, 4, and 5, Applicants have amended the claims to obviate these rejections. These amendments, namely replacing "characterized in that" with --wherein--, deleting "characterizing in that" and replacing with --comprising--, deleting "-type", or specifying a functional group capable of bonding to a biological substance, do not narrow the scope of the claims because they are merely made for clarification or make explicit what is inherent. In addition, the term "the" at line 1 of claim 1 has been replaced with --a-- and the term "one" has been inserted at line 4, of claim 13 for clarification. Therefore, these amendments do not narrow the scope of the claims.

Further with respect to the rejection at paragraph 4 of the action, Applicants enclose herewith a copy of pages 98-99 of Alberts et al., Molecular Biology of the Cell, 3rd Ed. (attachment 1), to demonstrate the specificity of the term "phosphodiester bonds". See particularly, page 98, last three lines, and page 99, Fig. 3-10(A).

With respect to the rejection at paragraph 1 of the action, Applicants have amended claim 1 to define a process comprising introducing a fluorescent conjugate comprising an oligonucleotide bonded to a rare-earth metal cryptate into the measuring medium. As such, Applicants respectfully submit that claim 1 is recited in a positive, active fashion. This is quite distinct from the cited case *Ex Parte Erlich*, 3 USPQ2d 1011 (Bd. Pat. App. And Int. 1986). At page 1017 of that case, the Board rejected claims 6 and 7 for merely reciting a use without any active, positive step delimiting how this use is actually practiced. In marked contrast, the present claims do not define a use but rather a process comprising introducing a fluorescent conjugate. Consequently, Applicants respectfully submit that this ground of rejection should be withdrawn. Moreover, breadth of a claim is not to be equated with indefiniteness (*In re Miller*, 441 F.2d 689,

169 USPQ 579 (CCPA 1971) and M.P.E.P. §2173.04).

With respect to the rejection at paragraph 3 of the action, the action alleges that claims 1-19 fail to correspond in a harmonization of nomenclature of which Applicants regard as the invention. Particularly, the Action alleges that the complexes become indefinite and confusing when switching back and forth between Eu-cryptate macrocyclic complex and a cryptate of many different rings or non-ring structure. Applicants respectfully traverse this ground of rejection.

Particularly, it is unclear what the Examiner is relying upon for this ground of rejection. Particularly, "regards" language of §112 may generally be relied upon to reject a claim "where some material submitted by Applicant, **other than his specification**, shows that a claim does not correspond in scope with what he regards as his invention". Emphasis added, *In re Conley*, 490 F.2d 972, 976, 180 USPQ 454 (CCPA 1974). See also *Solomon v. Kimberly-Clark Corp.*, 55 USPQ2d 1279, 1282 (Fed. Cir. 2000). Consequently, if the Examiner maintains this rejection, Applicants respectfully request that the Examiner particularly point out what materials submitted by Applicants show that a claim does not correspond in scope with what he regards as his invention.

Moreover, Applicants respectfully submit that the term "cryptand" is readily understood with sufficient particularity to one of ordinary skill in the art at the time the application was filed. Attached are definitions of cryptand and chelate from IUPAC Compendium of Chemical Terminology, Second Edition (1997). See Attachment 2. Cryptand is defined as a molecular entity comprising a cyclic or polycyclic assembly of binding sites that contains 3 or more binding sites held together by covalent bonds, and which defines a molecular cavity in such a way as to bind (and thus "hide" in the cavity) another molecular entity. The adduct thus formed is called a cryptate. In view of this definition, Applicants respectfully submit that one of ordinary skill in the art would understand the metes and bounds of the term "cryptate" with sufficient particularity.

With respect to the grounds of rejection at paragraph 6, the action alleges that the term "22" in claim 11 is not defined, the specification does not provide a standard for ascertaining the requisite degree, and thus one of ordinary skill in the art would not be reasonably apprised to the scope of the invention. In response, Applicants have amended claim 11 to recite nomenclature

referred in the article cited at page 3, lines 15-18 of the present specification. Particularly, the article J.M. Lehn in Struct. Bonding (Berlin), 16, 1, 1973 provides sufficient support for the amended terminology. Applicants respectfully submit that this amendment does not narrow the scope of claim 11 because it merely clarifies the terminology of the claim.

Thus, in view of the present claims, Applicants respectfully submit that the rejections under 35 USC §112, second paragraph be withdrawn.

Claim Rejections Under 35 USC 102

Claims 1-7, 9-11, 13, and 15-19 stand rejected as allegedly being anticipated by Li et al., *Bioconjugate Chemistry*, 1997, (8), pages 127-132 (Li). Particularly, the Action alleges that Li teaches that a process for reduced quenching may be attained in a fluorescence assay where a fluorescent conjugate comprising oligonucleotides bonded to a europium rare-earth metal cryptate is introduced into a medium. Applicants respectfully traverse these assertions.

Li discloses compounds comprising:

- a luminescent lanthanide chelate, namely DTPA (diethylenetriaminepentacetic acid);
- a chromophore moiety, namely, cs124; and
- a rare-earth metal (lanthanide) chelated by DTPA.

However, DTPA is not a cryptate.

Thus, Li does not disclose the method of the present invention because there is no cryptate included. On this basis alone, the rejection is untenable. Moreover, Li does not even involve a compound or method which allows the reducing of quenching. Although a cryptate is a type of chelate, there are no blazemarks or guideposts for one of skill in the art to pick a cryptate out of the countless other chelates that can be used or modified.

But, the invention as claimed relates to a method for reducing fluorescence quenching caused by the measuring media and not to conjugates themselves. Quenching of the emitted signal of the rare-earth metals generally designates an extinction of fluorescence.

This phenomenon may due to H_2O molecules, to other molecules which are present in the measuring medium, and particularly when that medium is a biological medium such as serum or a cell supernatant, or to intrinsic characteristics related to the chelate used. The quenching is

characterized by decrease of the signal emitted by the fluorophore, which correlates to a decrease in fluorescence lifetime.

In marked contrast, the present invention relates to a method which reduces the quenching of fluorescence caused by measuring medium. As depicted in Examples 3, 4, 8, 10 and 12, when carrying out the claimed method, the fluorescence's quenching which is usually observed in the presence of uric acid or of serum is significantly reduced. However, Table 1 of Li at page 130, left column, shows the fluorescence lifetimes of conjugates formed of [Eu]- or [Tb]-DTPA-single strand/double strand DNA, which have been synthesized by the dianhydride approach or by the isothiocyanate approach. The experiments are carried out in D_2O, which suppresses the quenching caused by H_2O. Therefore, Li discloses that:

> The Eu lifetime is relatively insensitive to environmental conditions, except for the well-known quenching effect of H_2O when lighted in the primary coordination sphere...

See paragraph **Luminescence Measurements**, *DNA complexes*, lines 6-9, at page 130. Thus, Li fails to suppress quenching by protecting the fluorescencing rare-earth metal from the measuring media. Rather, Li alters the measuring media, namely including D_2O to reduce quenching.

Thus, Li does not teach or suggest the claimed method, namely that fluorescence's quenching caused by the measuring medium can be lowered by using cryptate-nucleic acid conjugates.

Moreover, Li does not disclose any measurement in a biological medium, namely in a medium containing molecules other than H_2O and which may cause a quenching of fluorescence. Therefore, the information that europium conjugates are relatively insensitive in environmental conditions should be taken with restriction, as these "environmental conditions" only consist in temperature (5°C or room temperature), the solvent (H_2O or D_2O), the coupling technique and the hybridization of DNA to its complementary strand. Moreover, the experiments carried out with D_2O clearly show that the conjugates are indeed sensitive to water. Even though Li would teach that the disclosed conjugates are insensitive to H_2O molecules, Li fails to disclose a method

where the fluorescence quenching, due to a complex and uncontrolled medium, such as a biological medium, is significantly reduced. Consequently, Applicants respectfully submit that Li cannot anticipate the claimed invention.

Claim Rejections Under 35 USC §103(a)

Claims 1-9 stand rejected as allegedly being unpatentable over Li in view of U.S. Patent No. 5,985,563 (Hyl). Applicants respectfully traverse these rejections.

In view of the failings of the primary reference, the teachings of Hyl do not cure the basic deficiencies of Li. Consequently, their combination does not supply the missing teachings to render the claims obvious. So as not to burden the record further, Applicants will do not discuss Hyl in further detail except that Applicants do not necessarily acquiesce to any of the statements in the Office Action referring to such secondary reference, and reserve the right to comment later regarding the same, if ever necessary.

Supererogatorily, Li teaches that a fluorescent lifetime of Eu-DTPA conjugated to DNA is similar to that of unconjugated Eu-DTPA (page 130, left column, **Luminescence Measurements,** *DNA complexes*, lines 10-14), i.e. 2.48 ms vs. 2.41 ms. For Tb-DTPA conjugated to DNA, for lifetime is slightly is higher (3.063 vs. 2.63-see page 130, right column, lines 6-8). In marked contrast, when using the Eu-cryptates-DNA conjugates according to the invention, the lifetime is increased by nearly two times. See for example, Example, 1, page 13, line 8:0.60 ms and Example 3, page 17, line 10:1.1ms. This effect is not taught or suggested by Li. This data further demonstrates the patentability of Applicants' invention.

With respect to the teachings of the other secondary reference, U.S. Patent No. 5,162,508 (Lehn), because it does not cure the basic deficiencies of the primary reference, its combination with the other prior art would not supply the missing teachings to render the claims obvious. So as not to burden the record further, Applicants will not discuss each of the aforesaid secondary reference in detail except to state that Applicants do not necessarily acquiesce to any of the statements in the office action referring to such secondary reference and reserve the right to comment later regarding same, if every necessary.

In view of the above remarks, favorable reconsideration is courteously requested.

Attached hereto is a marked-up of the changes to the claims by the current Amendment. The attached page is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE." If there are any remaining issues which can be expedited by a telephone conference, the Examiner is courteously invited to telephone counsel at the telephone number indicated below.

The Commissioner is hereby authorized to charge any fees associated with this response or credit any overpayment to Deposit Account No. 13-3402.

Respectfully submitted,

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Attorney Docket No.: LOM 24

Date: **August 27, 2002**

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

Please amend claims 1-19 as follows:

1. (Amended) A process for reducing ~~the~~ a fluorescence quenching caused by ~~the~~ a measuring medium, in a fluorescence assay for an analyte using at least one fluorescent label, ~~characterized in that a fluorescent conjugate~~ comprising introducing a fluorescent conjugate comprising an oligonucleotide bonded to a rare-earth metal cryptate ~~is introduced~~ into the measuring medium.

2. (Amended) The process as claimed in claim 1, ~~characterized in that~~ wherein the oligonucleotide consists of a chain of ribonucleotide or deoxyribonucleotide units bonded to one another via phosphodiester ~~type~~ bonds.

3. (Amended) The process as claimed in claim 1, ~~characterized in that~~ wherein the oligonucleotide consists of a chain of ribonucleotide or deoxyribonucleotide units or of analogous units of nucleotides modified on the sugar or on the base and bonded to one another via natural phosphodiester ~~type~~ internucleotide bonds, some of the internucleotide bonds optionally being replaced with phosphonate, phosphoramide or phosphorothioate bonds.

4. (Amended) The process as claimed in claim 1, ~~characterized in that~~ wherein the oligonucleotide consists of a chain comprising both of ribonucleotide or deoxyribonucleotide units bonded to one another via phosphodiester ~~type~~ bonds and analogous units of nucleosides bonded to one another via amide bonds.

5. (Twice Amended) The process as claimed in claim 1, ~~characterized in that~~ wherein the oligonucleotide consists of ribonucleotide or deoxyribonucleotide units, one of which may comprise a functional group of NH_2, COOH, CHO, OH, SH, halide, sulfonate, epoxide, or maleimide, introduced onto or generated on said unit, or the functional group introduced using a

spacer arm bonded to the terminal phosphate group in the 3' or 5' position.

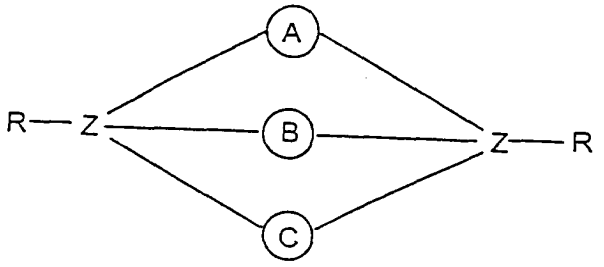
6. (Amended) The process as claimed in claim 5, ~~characterized in that~~ wherein said unit is the 5' terminal unit or 3' terminal unit.

7. (Twice Amended) The process as claimed in claim 1, ~~characterized in that~~ wherein the oligonucleotide comprises a chain of 5 to 50 nucleotides or a chain of 5 to 50 nucleotides and nucleotide or nucleoside analogs.

8. (Twice Amended) The process as claimed in claim 1, ~~characterized in that~~ wherein the oligonucleotide consists of a chain of ribonucleotide or deoxyribonucleotide units bonded to one another via phosphodiester-~~type~~ bonds and of analogous units of nucleosides bonded to one another via amide bonds, said oligonucleotide comprising at least 5 phosphodiester-~~type~~ internucleotide bonds at the end intended to be bonded to the cryptate.

9. (Twice Amended) The process as claimed in claim 1, ~~characterized in that~~ wherein the rare-earth metal cryptate is bonded covalently to the oligonucleotide either directly or via a spacer arm.

10. (Twice Amended) The process as claimed in claim 1, ~~characterized in that~~ wherein said rare-earth metal cryptate consists of at least one rare-earth metal salt complexed with a macropolycyclic compound of formula



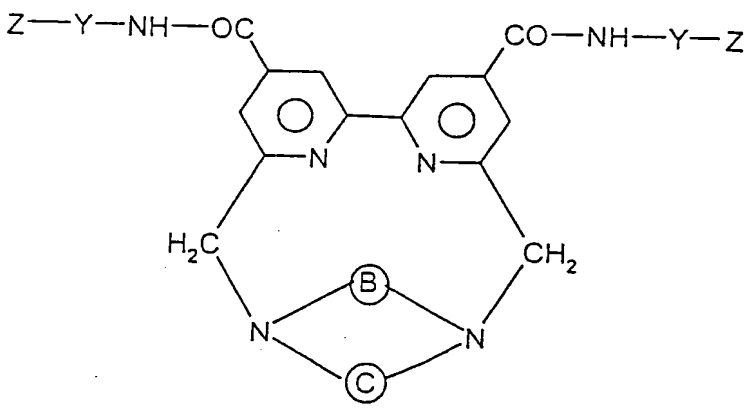
I

in which Z is an atom with 3 or 4 valencies, R is nothing or represents hydrogen, a hydroxy group, an amino group or a hydrocarbon-based radical, the divalent radicals Ⓐ , Ⓑ and Ⓒ are, independently of each other, hydrocarbon-based chains which optionally contain one or more hetero atoms and are optionally interrupted with a hetero macrocycle, at least one of the radicals Ⓐ, Ⓑ and Ⓒ , also comprising at least one molecular unit or consisting essentially of a molecular unit, said molecular unit having a triplet energy which is greater than that of the emission level of the complexed rare-earth metal ion.

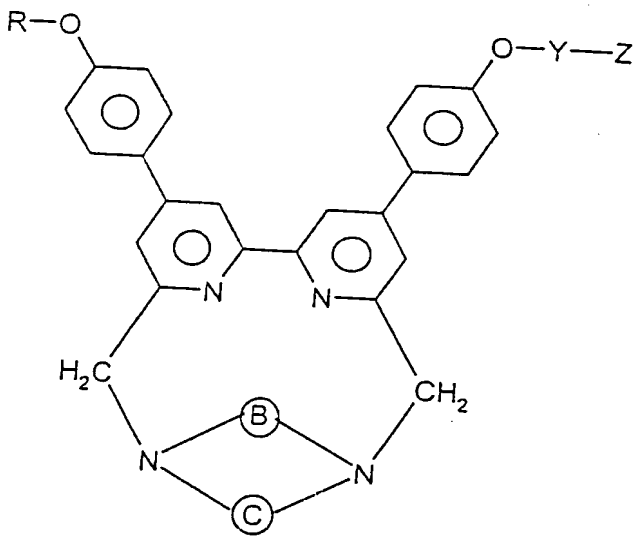
11. (Amended) The process as claimed in claim 10, ~~characterized in that~~ wherein the rare-earth metal cryptate consists of a rare-earth metal salt complexed with one of the macrocyclic or macropolycyclic compounds below:

[2.2.phenanthroline]; [2.2.phenanthroline amide]; [2.2.anthracene]; [2.2.anthracene amide]; [2.2.biisoquinoline]; [2.2.biphenyl-bis-pyridine]; [2.2.bipyridine]; [2.2.bipyridine amide]; ~~(22)phenanthroline; (22)phenanthrolinamide; (22)anthracene; (22)anthracenamide; (22)biisoquinoline; (22)biphenylbispyridine; (22)bipyridine; (22)bipyridinamide;~~ the macropolycycles trisbipyridine, trisphenanthroline, phenanthrolinebisbipyridine, biisoquinolinebisbipyridine, bisbipyridine diphenylbipyridine; a macropolycyclic compound comprising a molecular unit chosen from bipyrazines, bipyrimidines and nitrogen-containing heterocycles comprising N-oxide groups.

12. (Twice Amended) The process according to claim 1, ~~characterized in that~~ wherein the rare-earth metal cryptate consists of at least one rare-earth metal salt complexed with a macropolycyclic compound corresponding to one of the formulae II or III below:



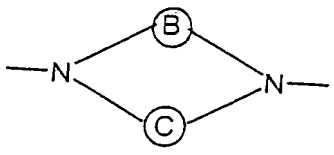
II



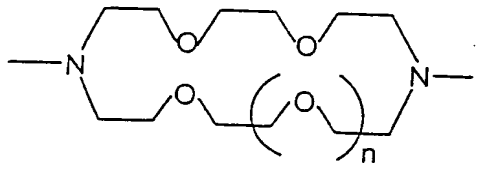
III

in which:

- the ring of formula



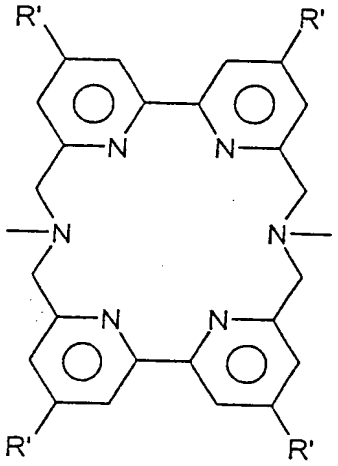
is one of the following rings:

1) 

n = 0 or 1

$[N_2O_4]$ macrocycle or cycle (22)

$[N_2O_3]$ macrocycle or cycle (21)

2) 

bisbipyridine macrocycle

-Y is a spacer group or spacer arm which consists of a divalent organic radical, chosen from linear or branched C_1 or C_{20} alkylene groups optionally containing one or more double bonds and/or optionally containing one or more hetero atoms such as oxygen, nitrogen, sulfur or phosphorus or one or more carbamoyl or carboxamido group(s); chosen from C_5 to C_8 cycloalkylene groups or chosen form C_6 to C_{14} arylene groups, said alkylene, cycloalkylene or arylene groups being optionally substituted with alkyl, aryl or sulfonate groups;

-Z is a functional group capable of bonding covalently to a biological substance;

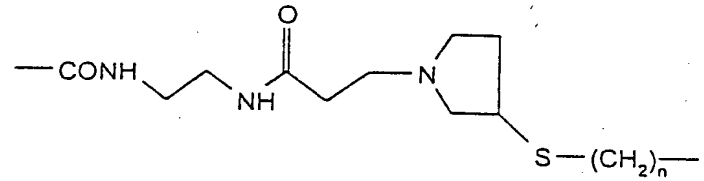
-R is a methyl group or represents the group -Y-Z;

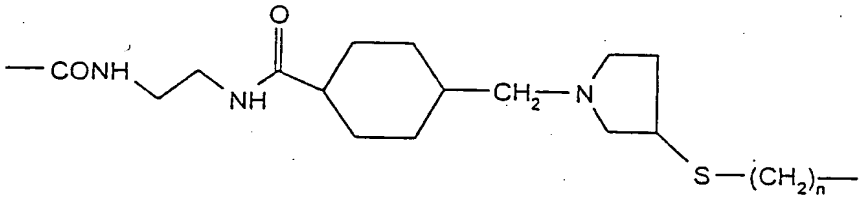
-R' is hydrogen or a group -COOR" in which R" is a C_1 to C_{10} alkyl group and preferably represents a methyl, ethyl or tert-butyl group, or alternatively R' is a group -CO-NH-Y-Z.

13. (Twice Amended) The process as claimed in claim 1, ~~characterized in that~~ wherein

the rare-earth metal cryptate is bonded to the oligonucleotide via a spacer arm consisting of a divalent organic radical chosen from C_1-C_{20} linear or branched alkylene groups optionally containing one or more double bonds or triple bonds and/or optionally containing one or more hetero atoms, such as oxygen, nitrogen, sulfur, phosphorus or one or more cabamoyl or carboxamino group(s); C_5-C_8 cycloalkylene groups and C_6-C_{14} arylene groups, said alkylene, cycloalkylene or arylene groups being optionally substituted with alkyl, aryl or sulfonate groups.

14. (Amended) The process as claimed in claim 13, ~~characterized in that~~ wherein the spacer arm is chosen from the groups:





in which n = 2 to 6, and -CONH-$(CH_2)_6$-, the attachment via the group -CONH taking place on the cryptate.

15. (Twice Amended) The method as claimed in claim 1, ~~characterized in that~~ wherein the rare-earth metal cryptate is a europium cryptate.

16. (Amended) The process as claimed in claim 15, ~~characterized in that~~ wherein the rare-earth metal cryptate is the europium cryptate Eu trisbipyridine or Eu [bisdiethoxybipyridine.bipyridine].

17. (Twice Amended) The process as claimed in claim 1, ~~characterized in that~~ wherein the fluorescent conjugate is used as the only label or as one of the fluorescent labels in the assay.

18. (Twice Amended) The process as claimed in claim 1, ~~characterized in that~~ wherein the fluorescent conjugate is bonded covalently to one of the members of a pair of molecules capable of binding specifically to one another, in particular a cellular receptor, an antigen, an antibody or a nucleic acid.

19. (Twice Amended) The process as claimed in claim 1, ~~characterized in that~~ wherein, in addition to said fluorescent conjugate, a fluorescent label comprising an acceptor fluorescent compound in the assay.